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Pb^{212}(ThB) Tracer Studies in Adult Beagle Dogs.* (24596)

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Interest in metabolism of Pb^{212}(ThB) developed from studies of chronic toxicities of Th^{228}(RdTh) and Ra^{228}(MsTh) in adult beagle dogs. In decay of these isotopes the inert gas thoron (Em^{220}) is formed, and, when decay occurs *in vivo*, a significant fraction of Em^{220} reaches the blood. Part is transported to the lungs and exhaled(1); the remainder decays to give Pb^{212}. Some Pb^{212} leaves the blood, but a significant amount binds to red cells to maintain a high concentration (relative to other activities in blood). For dosimetric purposes we need to know what fraction of Em^{220} decays in blood. This can be determined from measurements of Pb^{212} in blood, if we have a knowledge of Pb^{212} metabolism. There is an abundant literature on lead metabolism, but it relates mainly to chemical toxicity of lead, and includes only a limited number of studies in which carrier-free tracers were used. The affinity of red cells for tracer amounts of lead was reported as early as 1927(2) and the literature includes studies with rats(3) rabbits(4) and humans (5).

Methods. Two healthy young male adult beagle dogs from a large, well controlled colony were used. In Experiment A they received Pb^{212} by intravenous injection; in B, blood was drawn from each dog, tagged with Pb^{212} and reinjected into the dog from which it was drawn. Pb^{212} occurs in the Th^{228} decay chain and can be separated in high purity by de-emanation.

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$$Th^{228} \xrightarrow[1.9\ yr]{\alpha} Ra^{224} \xrightarrow[3.64\ d]{\alpha} Em^{220} \xrightarrow[54.5\ s]{\alpha} \xrightarrow{\alpha}$$

$$Pb^{212} \xrightarrow[10.6\ hr]{\beta,\gamma} Bi^{212} \xrightarrow[60.5\ m]{\beta,\gamma} \xrightarrow{\alpha} Pb^{208}$$

Air is bubbled through 1 to 2 ml Th^{228} solution in a small culture tube. The inert gas Em^{220} is carried by the effluent air through a short length of capillary tubing into a 1 L. flask. There it decays to Pb^{212} which deposits on the flask walls. As shown by Evans(6), a good yield of Pb^{212} is obtained if volume of the Th^{228} container plus connecting tubing (V_1) is small compared with the receiving flask (V_2), and if the air flow rate is such that V_1 is displaced in a time short compared with the half-period of Em^{220} (54.5 sec), but displacement of V_2 requires several half-periods. To prepare the injection solution for Exp. A, the flask was rinsed with 50 ml citric acid-sodium citrate buffer, pH = 3.5 and total citrate 0.08 M. In B, Em^{220} was bubbled through 10 ml heparinized blood for 1 hour, followed by 30 minutes incubation at 37°C. Since Pb^{212} was determined by gamma measurement, no chemical concentration or ashing of samples was necessary. Blood cell, plasma, and urine samples were measured in a well type liquid scintillation counter; the whole dog counter(7) was used for *in vivo* retention and feces measurements. In both cases sample radioactivity was compared with that of an aliquot of the injection standard. (This procedure automatically corrects for radioactive decay.) Each sample was measured over a period of time sufficient to demonstrate Pb^{212} - Bi^{212} equilibrium.

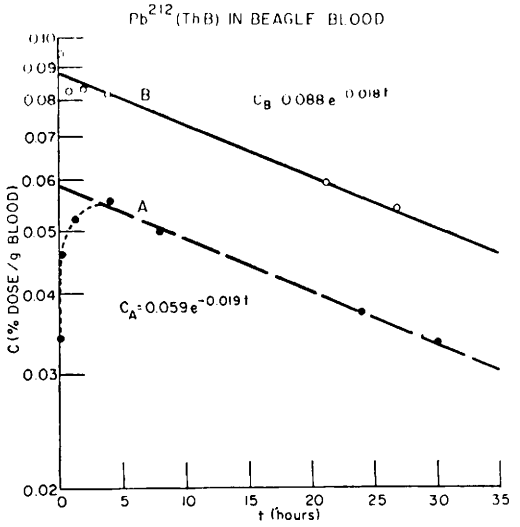

FIG. 1. Pb^{212} concentration in blood following I.V. inj., A, and inj. of tagged cells, B.

Results. Blood cells and plasma were measured separately, and in all blood samples 98% or more of the Pb^{212} was in the cellular fraction. Average values of Pb^{212} concentration in blood† during 30 hours after injection (3 Pb^{212} half periods) are shown in Fig. 1. Curve A illustrates the initial rapid rise in concentration and subsequent gradual decrease after intravenous injection of Pb^{212} in citrate solution. Curve B shows the gradual decrease in concentration which follows reinjection of blood which had been tagged with Pb^{212}. (Data were corrected for small amount of Pb^{212} in plasma.) The decreasing portion of curve A parallels curve B, and each can be described by a single exponential (C_A and C_B, Table I). Equations were calculated by method of least square using data for each dog and for both dogs. A biological half period of 37 hours for Pb^{212} in blood cells is calculated from C_A and C_B, which, when combined with the physical half period (10.6 hours), gives an effective half period of 8.2 hours.

Excretion and *in vivo* retention measurements are summarized in Table I. The 24 hour retention value of 91% agrees well with

† Volume of packed red cells was close to 0.50; hence, Pb^{212} concentration in cells is twice that in blood.

TABLE I. Blood Concentration and Excretion of Pb^{212}.

	Dog	Exp. A	Exp. B
		kg	
Dog wt.	1	10.6	10.4
	2	9.9	10.0
	Avg	10.2	10.2
		μc Pb^{212}	
Inj. dose	1	15	3.3
	2	15	3.9
	Avg	15	3.6
		% dose/g blood, hr	
Blood conc.	1	C_A=.064e$^{-.022t}$	C_B=.088e$^{-.021t}$
	2	C_A=.053e$^{-.015t}$	C_B=.088e$^{-.015t}$
	Both	C_A=.0588e$^{-.0187t}$	C_B=.0883e$^{-.0181t}$
		hr	
Biological $t_{1/2}$ for Pb^{212} in blood cells*		37.1	37.6
Effective $t_{1/2}$ for Pb^{212} in blood cells*		8.24	8.27
		% dose	
In vivo retention (24 hr)	1	92.1	
	2	90.1	
	Avg	91.1	
Total excretion (24 hr)	1	3.2	
	2	4.4	
	Avg	3.8	
Idem (30 hr)	1	3.8	5.5
	2	4.7	6.2
	Avg	4.3	5.9
Urinary/fecal excretion	1	6.1	3.4
	2	4.5	3.6
	Both	5.1	3.5

* Both dogs.

the 96% calculated from excretion. We measured an excess of Bi^{212} over Pb^{212} in urine in amount such that *in vivo* retention is estimated to be 1 to 2% low, while retention determined from excretion is more likely to be high. The two 30 hour excretion values also agree well. Predominance of urinary over fecal excretion is of limited significance, for the half period of Pb^{212} is short compared with lag in fecal excretion. Values (5.1 and 3.5) shown in Table I are therefore not representative of beagle lead excretion in general, and describe only excretion of short lived Pb^{212}.

Discussion. When blood cells are tagged *in vitro* and then reinjected, blood cell Pb^{212} decreases continuously with time, but when Pb^{212} is injected into the blood, Pb^{212} in cells increases to a maximum (which is ⅔ of the tagged cell value at that time), then decreases

at the same rate (Fig. 1). To explain this, let us consider blood cells (BC), blood plasma (Pl), and 2 extravascular compartments (EVI and EVII), the second of which includes excretion, which are related as follows:

$$BC \underset{2}{\overset{1}{\leftrightharpoons}} Pl \underset{4}{\overset{3}{\rightleftharpoons}} EVI \underset{6}{\overset{5}{\rightleftharpoons}} EVII$$

That blood cells have a much greater affinity for lead than does plasma is demonstrated by the fact that introduction of tracer amounts of lead into whole blood results in 98% or more in the cellular fraction. Schubert and White (3) have shown that the number of cellular binding sites for lead atoms is limited, but that the limit is reached only when mg amounts of lead are used. Immediately after injection of Pb^{212}, concentration in Pl is high and Pb^{212} diffuses into EVI and binds to BC (paths 1 and 3). Depletion of Pl leads to a reversal of the concentration gradient between Pl and EVI very soon after injection, and, since transfer between Pl and EVI is rapid, Pb^{212} returns from EVI to Pl where it is again available to BC, and Pb^{212} in BC continues to increase. At the same time Pb^{212} is also moving from EVI to EVII. This 2-way depletion of EVI continues until the concentration gradient between EVI and Pl reverses again. At the time of this second reversal Pb^{212} in BC has reached its maximum value (65%), it is low in Pl and EVI, and the remaining 35% is mainly in EVII. In Fig. 1, this time corresponds to the maximum in A; thereafter, both A and B represent slow transfer of Pb^{212} from BC to EVII along paths 2, 3, and 5. Since the observed biological half-life for Pb^{212} in cells (37 hours) is long compared with the physical half-life, slow return on path 6 can be neglected (which permits inclusion of excretion in EVII).

From the intercept of B in Fig. 1 and blood specific gravity = 1.06, blood volume is calculated to be 104 ml blood/kg. Parkinson and Dougherty(8) determined red cell and plasma volumes simultaneously, using Cr^{51} tagged red cells and I^{131} tagged human albumin, for 21 normal dogs from the same colony. They found an average blood volume of 94.4 ($\pm$ 16.6) ml/kg. Our Pb^{212} tagged cell value lies within one standard deviation of their more accurately determined value.

Using the longer lived isotope Pb^{210} Schubert and White(3) found a half period for Pb^{210} in rat blood cells of 30 hours, which is close to our dog value of 37 hours. Following intravenous injection of carrier-free Pb^{210} they report a maximum of 10% in rat blood cells at 25 minutes in contrast with the maximum dog value of 65%.

Hevesy and Nylin(5) used Pb^{212} to determine blood volumes in humans. They report a loss of Pb^{212} from blood cells of less than 4% per hour, from which we calculate 17 hours as a minimum value for the biological half-life of Pb^{212} in human blood cells.

In decay of Pb^{212} to stable Pb^{208} there are 2 beta and one alpha disintegrations plus accompanying gamma radiation. Total energy is 10.1 Mev (8.0 Mev α, 0.83 Mev β, and 1.3 Mev γ). In complete decay of 1 μc Pb^{212} plus daughters, total alpha energy is 2.6×10^4 ergs. How is this energy divided among blood, remainder of dog, and excreta?

First, we must consider radioactive equilibrium for we observed an excess of Bi^{212} over Pb^{212} in some urine samples representing 1 to 2% of the total Bi^{212}. In measuring Pb^{212} in blood cells, there is a lapse of about 20 minutes from time blood is drawn to time of the first count. Within this limitation we observed no disequilibrium, and assumption of radioactive equilibrium probably introduces only a small error. Assuming equilibrium, alpha radiation dose to blood for experiments A and B can be calculated using the equations for C_A and C_B (Table I). (In using C_A for Exp. A, we overestimate during the initial period when Pb^{212} in cells is increasing, but calculation shows this error to be only 2 to 3%.) When Pb^{212} is injected intravenously, alpha dose to blood is $\dfrac{8.0 \times 1.6 \times 10^{-6} \times 3.700 \times 10^4 \times 60 \times 60 \times A}{100}$ $\int_0^\infty 5.88 \times 10^{-4} e^{-.0841t}\, dt = .12$ A rads, where t is in hours and A = μc Pb^{212} injected. The fraction of Pb^{212} atoms which decay in blood is $.667 \int_0^\infty e^{-(.0187 + .0654)t}\, dt \Big/ \int_0^\infty e^{-.0654t}\, dt =$

.52. In the tagged cell experiment, alpha dose to blood per μc Pb^{212} is .18 rad, and fraction of Pb^{212} atoms which decay in blood is .78.

At 30 hours Pb^{212} in excreta is about 6% of the total Pb^{212} at that time. Most of the excretion takes place 1 to 3 half periods after injection, so that the actual amount of Pb^{212} which decays outside the dog (*i.e.*, in excreta) is only 1 to 2% of the amount originally injected. Thus our error will be small if we use the simplifying assumption that all Pb^{212} decays inside the dog. Hence, in summary, when blood cells are tagged with Pb^{212} and injected into the dog, about 4 5 of the alpha energy is delivered to blood and 1 5 to the remainder of the dog. When Pb^{212} is injected intravenously, half decays in blood and half in the rest of the dog. It follows then, that when thoron decays in blood, half the resulting Pb^{212} can be expected to decay in blood and half elsewhere in the dog. Therefore, with information gained from these experiments, we will be able to calculate the amount of thoron which decays in blood if we know Pb^{212} concentration in blood and total blood volume.

Summary. Beagle metabolism of short-lived Pb^{212} has been studied following intravenous injection, and after transfusion of blood cells tagged with Pb^{212} *in vitro*. The latter proved to be a satisfactory method to determine blood volume. When Pb^{212} was given intravenously, *in vivo* tagging of blood cells occurred. A maximum of 65% of the activity in cells was reached at 2 to 3 hours after injection, then Pb^{212} in blood cells decreased with a biological $t_{1/2}$ = 37 hours and an effective $t_{1/2}$ = 8.2 hours. The same decrease occurred after injection of *in vitro* tagged cells. In both experiments essentially all Pb^{212} decayed inside the dog. Half decayed in blood in the *in vivo* tagging experiment; 4 5 decayed in blood when tagging was done *in vitro*.

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Isolation of Asian Virus from Extrapulmonary Tissues in Fatal Human Influenza.*† (24597)

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Thirty-three influenza-associated deaths were observed in greater Cleveland during the 1957 pandemic of Asian influenza. Asian virus was isolated from lung or trachea in 25 of the 33, and from extrapulmonary tissues in 3 of the 25 respiratory tract-positive cases. Al-

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